Docket No.: INTERLINK 3.0-003
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Everett et al.

Application No.: 09/431,546

Group Art Unit: 1635

Filed: October 29, 1999

Examiner: S. McGarry

For: PEPTIDES WITH ENHANCED STABILITY TO PROTEASE DEGRADATION

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
Washington, DC 20231

Dear Sir:

I, Nicholas P. Everett, do declare as follows:

1. I am Vice President of InterLink Biotechnologies, LLC, a co-assignee of this patent application, a position I have held since the inception of the company. I received a Ph.D. in plant biochemistry and physiology from the University of Leicester, England in 1978. I have authored or co-authored more than 20 publications, and given more than 50 presentations in the fields of plant biotechnology, antimicrobial peptides and drug discovery.

2. I am a co-inventor of this patent application. I am familiar with the correspondence with the Patent Office to date, including the Office Communication mailed March 22, 2002.

3. The purpose of this Declaration is to present evidence generated from two separate experiments conducted under my direct supervision and, in part, with my own hands. The data generated from the first experiment demonstrate that indolicidin exerted a protective effect to a second peptide against protease degradation. Specifically, the data set forth below show that the degree of cross-protection of a magainin derivative by indolicidin was nearly identical to the cross-protection provided by Rev4 In the second experiment, Rev4 was shown to stabilize a magainin peptide that had been applied to leaf tissue from protease degradation.

4. In one experiment, assays for WCE protease degradation were performed similarly to the protocol described in Example 9 of the patent application except for slight differences in the growth of the tobacco plants and the particular magainin peptide cross-protected. In this experiment the tobacco proteases were obtained from young plants grown under sterile conditions in a growth chamber, instead of more mature plants growing in soil in a greenhouse. The magainin peptide used was a substitution derivative of magainin known as pexiganan (GIGKFLKKAKKFGKAFVKILKK , Ge et al. 1999. Antimicrob. Agents Chemother. 43(4):782-8) instead of magainin 2 (GIGKFLHSAKKFGKAFVGEIMNS). Incubation mixtures contained 20% by volume WCE from tobacco leaves, 200 micromolar concentrations of each peptide, and the amounts of peptides remaining in the mixture after various incubation times were determined by HPLC, as previously described. The data set forth below show that indolicidin was able to cross-protect pexiganan against WCE protease degradation equivalent to that provided by Rev4.

Peptides	Percentage Pexiganan Remaining			
	0 hours	4 hours	8 hours	21 hours
Pexiganan alone	100	76	62	43
Pexiganan + Rev4	100	100	100	90
Pexiganan + Indolicidin	100	100	100	93

5. In the second experiment, ten nmoles of each peptide were applied to the upper leaf surface of leaf discs cut from tobacco plants grown under sterile conditions, and the leaf discs incubated in a humidified, illuminated growth chamber. After the times indicated, the remaining peptides were extracted from the leaf discs with 25% v/v acetonitrile (ACN) containing 1% v/v trifluoroacetic acid (TFA) and subjected to HPLC analysis. The original peptide peak was integrated and the average values from 3 replicates were calculated. The averages were converted into percentage peptide remaining using the zero time point as reference (100%). The data shown in the table below demonstrate that pexiganan (GIGKFLKKAKKFGKAFVKILKK) alone is completely degraded within 48 hours of application to tobacco leaf tissue but, in the presence of an equimolar amount of Rev4, approximately 35% of the pexiganan remains intact.

Treatment	Pexiganan Remaining Intact (%)	
	0 Hours Incubation	48 Hours Incubation
Pexiganan alone	1318 (100)	0 (0)
Pexiganan + Rev4	1228 (100)	427 (35)

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In view of these data, it is my opinion that Rev4 would also exhibit similar protective activity to a second peptide against protease inhibitory activity if it were the expression product of a transgene in a transgenic plant.

I declare under penalty of perjury that the foregoing is true and correct. I further state that I have been warned that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and such willful false statements may jeopardize the validity of the application or any patent resulting therefrom. I state that all statements made of my own knowledge are true and all statements made on information and belief are believed to be true.

Dated: 4 November 2002

Nicholas P. Everett

NICHOLAS P. EVERETT

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